STUDIES ON A CORTICAL LAYER RESPONSE TO STIMULATING AGENTS IN THE ARBACIA EGG

III. Response to Non-Electrolytes

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Introduction

According to Moore (1930*a*, 1930*b*), and Moore and Moore (1931), eggs of *Strongylocentrotus purpuratus, Dendraster eccentricus,* and *Paracentrotus lividus,* treated with non-electrolyte solutions isosmotic with sea water, are rendered incapable of " forming " fertilization membranes upon subsequent insemination in sea water. Moore suggested that this loss of ability to elevate a membrane was " the result of the diffusion of something from the egg which renders membrane formation impossible." Moreover, the " power of forming membranes once lost cannot be regenerated by the egg." In his experiments Moore generally used molar urea solution, pH 7, although similar results were obtained with both glycerine and sucrose solutions. Chase (1935) reported evidence in confirmation of Moore's results on *Strongylocentrotus purpuratus,* and *Dendraster eccentricus.*

In the first two papers of the present series (Moser, 1939*a*, 1939*b*) it was shown that stimulation of the *Arbacia* egg results in the breakdown of a thin layer of cortical granules. It was postulated that vacuoles arising from the breakdown of the cortical layer granules play a part both in the elevation of the fertilization membrane as well as in the other related visible cortical changes which occur at the stimulated egg surface. It was suggested further, in terms of the theory of stimulation and response advanced by Heilbrunn and his students (Heilbrunn and Daugherty, 1933; Heilbrunn, 1937), that an initial rapid, and doubtless invisible cortical response led not only to the above-mentioned visible phenomena at the egg surface, but also to the colloidal changes associated with cleavage. According to this concept, membrane elevation and the processes leading to segmentation are related only in that both depend upon a common reaction. It is therefore conceivable that this reaction, which, presumably, is common to both membrane elevation and cleavage

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might initiate either response without calling forth the other. Essentially in accord with this idea are Glaser (1913), Loeb (1915), Lillie (1919), Gray (1922), Just (1919*a*, 1923), and Carter (1924).

If, now, it be assumed that the above theory is correct, and in addition that the forms with which Moore has worked represent systems which are comparable to the *Arbacia* egg and also that the loss of membrane "forming" ability is the result of the diffusion of some substance from the egg, then the failure of the membrane to elevate in urea-treated eggs, may be attributed to the loss of a substance which initiates the visible cortical phenomena. But if this be true, then how are we to explain the fact that cleavage takes place when these eggs are inseminated, for according to the theory both the visible cortical response and segmentation are initiated by the same substance? With this problem in mind, experiments similar in some respects to those of Moore have been performed.

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Material and Methods

Arbacia eggs and dry sperm were secured in the usual manner.

A molar urea solution, pH 6.8 (indicator method), was used in most of the experiments. In a few cases, molar solutions of thiourea, glycerine or sucrose were used.

The procedure of exposing *Arbacia* eggs to non-electrolyte solutions was somewhat similar to that of Moore (1930*a*). One or 2 cc. of a thick suspension of eggs was pipetted into 50 cc. of the non-electrolyte solution. The eggs were exposed to the action of this solution for 3–5 seconds, 30 seconds, 1, 1½, 2 and 2½ minutes. Whenever the treatment with non-electrolyte lasted for more than one minute, the eggs were transferred to a second 50 cc. of the same non-electrolyte at the end of the first minute. After exposure to the non-electrolyte solution, the eggs were transferred to sea water and divided into two lots, one of which was inseminated. It is perhaps needless to say that the necessary precautions were followed in order not to contaminate the uninseminated lot with sperm cells.

In order to study the effect of an agent upon the egg cortex in terms of the breakdown of the cortical layer granules, it is necessary to shift the underlying endoplasmic granules to one side of the egg. This was accomplished by means of an electric centrifuge which developed a force

of approximately 6,000 times gravity. When the eggs are centrifuged until they show five distinct strata, the granular cortex becomes perfectly visible over the clear hyaline zone. The effects of non-electrolyte solutions on the egg cortex were observed by placing a drop of the centrifuged eggs and several drops of the non-electrolyte solution side by side on a glass slide which had previously been fixed in position on the stage of the microscope. The non-electrolyte solution was then made to flow into the drop of eggs and the visible effects noted.

During the period when these experiments were made, room temperature ranged from 21.0° to 27.0° C.; for any one set of experiments the temperature remained fairly constant.

Observations and Results

Cleavage and Membranes of Urea-treated Eggs

The results obtained in one set of experiments, after treatment of *Arbacia* eggs with molar urea solution for various time intervals, are summarized in Table I. Upon examining Table I it becomes apparent that molar urea solution acts as a parthenogenetic agent. Since cleavage occurs at a relatively slow rate after activation with non-electrolyte solutions, the eggs were counted from 4½ to 6½ hours after treatment. The percentages given in the tables are based upon counts of one hundred or more eggs. Cleavage in the uninseminated and the inseminated lots of eggs is quite irregular, except in those cases among the inseminated eggs which have given rise to blastulae. Generally no more than eight or nine blastomeres develop in any one case (blastulae excepted), and usually the number is less.

Microscopic examination of the irregularly cleaving eggs which have been exposed to urea for from ½ to 1½ minutes, reveals that nearly all of these eggs possess thin fertilization membranes. The degree of thinness of the membrane seems to be a function of the length of time that the eggs have been treated with urea. Thus, in those eggs which were treated for ½ minute, the membranes are more easily discerned than those of eggs which had been exposed for 1 minute, and these latter membranes can be seen more easily than those of eggs treated for 1½ minutes. In other experiments in which the eggs had been exposed for from 3–5 seconds, apparently normal membranes were visible when these eggs were examined shortly thereafter in sea water. Indeed, eggs treated for such short time intervals cannot be distinguished from normally inseminated ova. After exposures of two or more minutes usually no vestige of the membrane can be seen.

In addition to the fact that fertilization membranes may be seen in

both the inseminated and the uninseminated lots of eggs after treatment with urea for time intervals of from ½ to 1½ minutes, it may be of interest to point out that these membranes are rather closely applied to the egg surface. In some cases such membranes can be identified only in those areas where they stretch between adjacent blastomeres. This is especially true for exposures of 1½ minutes.

The blastulae which develop in the inseminated lot of eggs after treatment for more than 1½ minutes do not appear to have fertilization membranes. The normal relationships of the cells making up the blastulae are, however, retained. Possibly a very thin remnant of the hya-

TABLE I

Percentage of cleavage and of blastulae in uninseminated and inseminated lots of eggs after treatment with molar urea solution for various time intervals. The number of blastulae is given in terms of the percentage of eggs cleaving rather than in terms of the total number (cleaving plus non-cleaving) of eggs. (Room temperature 23.0° C.)

Time of exposure to molar urea solution	Uninseminated lot		Inseminated lot	
	Percentage cleavage	Percentage blastulae of those cleaving	Percentage cleavage	Percentage blastulae of those cleaving
minutes	*per cent*	*per cent*	*per cent*	*per cent*
0.5	83	0	90	5
1.0	93	0	96	10
1.5	96	0	96	36
2.0	95	0	96	33
2.5	94	0	93	28

Fertilized untreated controls 94 per cent blastulae, 5 per cent in early cleavage, all with membranes. Unfertilized untreated controls 100 per cent without membranes and non-cleaving.

line plasma membrane holds these cells together. In occasional experiments, when the treatment with non-electrolyte solution lasted for two or more minutes, the blastomeres of both the uninseminated and the inseminated lots of eggs become separated; only in one or two exceptional cases did this occur with exposures of 1½ minutes.

Fertilization Superposed upon Incomplete Artificial Activation

The percentage of cleavage in the uninseminated and the inseminated lots of eggs (Table I) is very nearly the same. Now, since the percentage of blastulae among the uninseminated lot of eggs is zero, while the percentage of blastulae in the inseminated lot is quite high, it would seem that fertilization has been superposed upon incomplete artificial

activation. It is evident, moreover, that after exposure to urea of ½ to 1 minute, the percentage of blastulae is considerably lower than that which occurs after exposures of from 1½ to 2½ minutes. One might have expected that just the reverse would be true; that with the shorter treatment with urea, the percentage of superposed fertilization would be greater. It will be remembered, however, that the fertilization membrane is most easily seen after the shorter intervals of exposure to the non-electrolyte solution. It would seem possible, therefore, that the relatively low percentage of blastulae found among the cleaving eggs of the inseminated lot, after treatment for ½ to 1 minute, is due to the fact that the membrane may act as a block to the entrance of sperm cells in these instances, while with the longer exposures this block is partially or completely removed.

However, it must not be assumed, as Table I might lead one to believe, that the percentage of cleavage in the uninseminated and inseminated lots of eggs always approximate each other. If among the uninseminated eggs it is evident that a certain percentage of the eggs is dividing, then it may be expected that the same or a higher percentage of eggs will be dividing in the inseminated lot. Thus in Table II the value 55 per cent cleavage, in the uninseminated lot, after treatment with urea for 3–5 seconds, approximates 54 per cent cleavage in the inseminated lot. Likewise, after exposure to urea of 0.5 minute, the value 85 per cent cleavage, in the uninseminated lot of eggs, is very near to that of 86 per cent in the inseminated lot. However, the next two values, 97 per cent and 95 per cent, in the inseminated lot of eggs, are significantly higher than the corresponding percentages in the uninseminated lot. This increase in the percentage of cleavage in the inseminated eggs as compared to the uninseminated eggs is probably due to the activation of eggs which with the urea treatment alone would not have divided.

Fertilization (Urea-activated) Membrane as Block to Insemination

A trend which was indicated in the column of figures under the heading " Percentage blastulae of those cleaving " in the inseminated lot of Table I is more definite in the same column of Table II. That is, as the time of exposure to the non-electrolyte solution increases, there is a definite tendency for the percentage of blastulae among the cleaving eggs of the inseminated eggs to increase. This, as earlier pointed out, is doubtless due to the presence of the fertilization membrane, which becomes less and less of a block to the entrance of spermatozoa as the exposure time to the non-electrolyte increases. For example, the figure zero under the heading " Percentage blastulae of those cleaving " in the

inseminated lot of eggs of Table II is perhaps especially significant, for it will be remembered that exposures of 3–5 seconds produce eggs whose membranes very closely simulate those of normally fertilized ova. Moreover, the increasing percentages, 69 per cent, 87 per cent, and 91 per cent after exposures of 0.5, 1.0, and 2.0 minutes respectively, are indicative, since the membranes become thinner and finally disappear as the exposure time increases.

In exceptional batches of eggs the urea had no effect other than to initiate the reaction leading to the elevation of the membrane. Thus in such an instance, exposures of 3–5 seconds, 0.5 minute, 1.0 minute and 2.0 minutes yielded no cleavage in the uninseminated lot of eggs,

TABLE II

Percentage of cleavage and of blastulae in uninseminated and inseminated lots of eggs after treatment with molar urea solution for various time intervals. The number of blastulae is given in terms of the percentage of eggs cleaving rather than in terms of the total number (cleaving plus non-cleaving) of eggs. (Room temperature 25.0° C.)

Time of exposure to molar urea solution	Uninseminated lot		Inseminated lot	
	Percentage cleavage	Percentage blastulae of those cleaving	Percentage cleavage	Percentage blastulae of those cleaving
	per cent	*per cent*	*per cent*	*per cent*
3–5 sec.	55	0	54	0
0.5 min.	85	0	86	69
1.0 min.	85	0	97	87
2.0 min.	81	0	95	91

Fertilized untreated controls 100 per cent blastulae. Unfertilized untreated controls 100 per cent without membranes and non-dividing.

except after the 0.5 minute interval; segmentation occurred in only 1.8 per cent of these latter eggs. In the inseminated lot, however, as many as 93 per cent of the eggs gave rise to blastulae; clearly as a consequence of the added stimulus of fertilization.

Direct Observations on Response of Egg Cortex to Urea

From the observations already presented in this paper, it is evident that the shorter exposures of *Arbacia* eggs to molar urea solutions result in the elevation of a fertilization membrane, which is readily visible when the eggs have been placed in sea water. After the longer exposures, it is impossible to see the fertilization membrane. Relative to these facts, a number of possibilities suggest themselves. In the first place the short exposures might initiate a change at the egg surface which

would result in the breakdown of the cortical layer granules (see Moser, 1939*a*, 1939*b*) only after the eggs have been placed in sea water. According to this concept, the longer exposures would in some way inhibit the cortical response when the eggs are subsequently returned to sea water. As a second possibility, the cortical layer granules might break down and membrane elevation occur during the period of exposure to the non-electrolyte. If the latter possibility be true, then after the longer exposures, it is impossible to see the fertilization membrane, not because membrane elevation has been inhibited, but because it has disappeared subsequent to the process of elevation.

Earlier observations have suggested that the latter of the above possibilities is the correct one. The results which follow furnish absolute proof in this direction. Thus, when four to five drops of a molar urea solution were made to flow into a drop of centrifuged or uncentrifuged eggs, the cortical layer granule breakdown response was observed. This response to urea is essentially the same as that obtained in the presence of sperm cells (Moser, 1939*a*) and other stimulating agents (Moser, 1939*b*; *see* also Moser, 1937, for preliminary report on the effect of urea). The initial visible cortical changes are wave-like in both the centrifuged and uncentrifuged eggs. The direction of the wave apparently depends upon the direction of flow of the non-electrolyte solution. For example, when the urea flowed into the drop of eggs from the right side of the microscopical field, then the visible cortical response progressed from right to left over the egg surface as viewed under the microscope.

Generally, immediately following the breakdown of the cortical layer granules, rapid elevation of the fertilization membrane occurs. In many cases the perivitelline space is from two to three times as wide as in normal fertilization. If at this stage, or immediately after the initial visible cortical response the eggs are placed in sea water, then the membranes appear to be almost normal, although they may be somewhat thinner and further removed from the egg surface. In the event that the eggs are not removed to sea water immediately after membrane elevation, the membranes begin to recede toward the egg surface and gradually become thinner, until finally in most cases no vestige of the membrane can be seen. If these eggs are then placed in sea water, many of them exhibit the early cleavage stages. The cleavages are very irregular and in most cases do not exceed eight or nine cells. Sometimes the blastomeres are held together by means of a thin film which is probably the hyaline plasma membrane, while in other instances the blastomeres are strung out somewhat in the manner of a colony of yeast cells.

Most of the above-described observations have been made on eggs treated with molar urea solutions. Similar results have been obtained in response to molar solutions of thiourea, glycerine, and sucrose.

Amoeboid Activity of Urea-treated Eggs

It may be of some interest to point out that eggs which are not removed from the urea solutions do not cleave, though they do exhibit a form of protoplasmic activity which may be but a variation of the normal cleavage process. Thus, shortly after *Arbacia* eggs have been placed in urea solution, they not only lose their spherical shape, but indeed exhibit a peculiar form of amoeboid movement. Characteristically a rather large portion of the surface of the egg suddenly rushes outward followed immediately by endoplasmic material which flows into and with the outward moving surface layer. Generally no two such bursts of movement succeed each other at the same portion of the egg. A second burst of movement may occur at a point immediately adjacent to the previous one, or anywhere between the preceding locus of movement and the opposite pole of the cell. For the most part this rather random activity of amoeboid *Arbacia* eggs results in rotatory movement alone, though in some instances translatory movement over short distances does occur.

After the eggs have been in a molar urea solution for approximately two hours, clear blebs and vesicles begin to form at their surfaces. These vesicles, which in many instances become separated from the eggs, vary from a few microns in diameter to a size which approaches that of the egg diameter itself. Concurrently with the formation of the clear vesicles, the amoeboid activity of the eggs becomes decidedly slower, and during the next hour movement ceases entirely.

Discussion

The observations presented in this paper make it possible to select some data for which both the experimental procedure and the results parallel those of Moore (1930*a*, 1930*b*), and Moore and Moore (1931). Thus when eggs are treated with molar urea solution for two or more minutes and are then transferred to sea water in which they are subsequently inseminated, cleavage is found to take place as well as the development of rather normal looking blastulae. Moreover, these dividing cells do not possess a fertilization membrane, though they may be held together by a thin remnant of the hyaline plasma membrane. From such data alone, one might readily conclude that treatment with molar urea solution results in the inhibition of fertilization membrane elevation even though the processes of fertilization and cleavage still occur.

Such a conclusion becomes untenable, however, in the light of other observations and results, for it has been shown that non-electrolyte solutions initiate not only the visible phenomena at the surface of the egg but the process of cleavage as well. The fact that one cannot see the fertilization membrane upon insemination after a two-minute treatment with urea is not a consequence of the inhibition of membrane elevation, but rather it is due to the fact that membrane elevation has been initiated and the membrane then dissolved by the very substance responsible for the reaction leading to its elevation.

It is possible that the forms with which Moore (1930) has worked represent systems which differ from the Arbacia egg. If, however, it be true that these forms are similar, then the fact that membrane elevation was not obtained upon insemination after treatment with urea in the case of *Strongylocentrotus purpuratus, Dendraster eccentricus,* and *Paracentrotus lividus,* might be explained by assuming that here, as in the *Arbacia* egg, membrane elevation and the dissolution of the membrane had already taken place in the urea solution. Thus the question of how non-electrolyte solutions prevent membrane elevation either irreversibly or otherwise completely loses significance. Moreover, the blastulae, however plate-like, obtained in the above-mentioned forms, may have been the result of fertilization superposed upon incomplete artificial parthenogenesis. In agreement with the concept of the superposition of fertilization upon incomplete artificial activation are the papers of C. R. Moore (1916, 1917), Just (1919*b*, 1922), and Lillie (1921). However, these workers have presented evidence which indicates that membrane elevation in itself is the result of a complete cortical response.

Again with regard to the elevation of the fertilization membrane, Motomura (1934, 1938) has shown that eggs of *Strongylocentrotus nudus, Strongylocentrotus pulcherrimus* and *Strongylocentrotus depressus* elevate membranes when placed in sea water or simple salt solutions after previous treatment with molar urea for from four to ten seconds. Thus, he (Motomura, 1938) says, " When eggs of a sea urchin are previously activated with urea solution, they form the fertilization membrane parthenogenetically even in simple salt solutions of monovalent and divalent cations." According to this concept, the membrane does not elevate in the urea solution itself, but is conditioned by a second treatment with sea water or " simple salt solution." With these forms, as with the Arbacia egg, it is likely that membrane elevation occurs in the urea solution and that the dissolution of the membrane is prevented when the eggs are returned to sea water or the " simple salt solutions." This seems especially possible in view of Motomura's (1934) conclusion,

" The loss of capacity of membrane formation can be caused by treating the eggs for a long time either with isotonic urea solution or with butyric acid sea water."

Amoeboid movements of egg cells have previously been described. Thus, Lillie (1902) and E. B. Harvey (1939) have shown that the eggs of *Chaetopterus pergamentaceus* exhibit such activity under certain experimental conditions. Churney (1940) describes amoeboid movements of fertilized *Arbacia* eggs treated with "pure solutions of potassium magnesium." While an explanation of the amoeboid activity of *Arbacia* eggs treated with urea will not be attempted, yet the phenomenon is of some special interest since it occurs in cells whose normal function is to divide. Indeed these movements may be an abortive form of cleavage furrow formation, an idea which falls in line with the concept, advanced by Marsland (1936, 1938, 1939) and Schechtman (1937), that cleavage furrow formation and amoeboid movement are but two expressions of the same general type of protoplasmic activity.

Summary

1. *Arbacia punctulata* eggs treated with molar concentrations of non-electrolyte solutions (urea, thiourea, glycerine or sucrose) exhibit essentially the same kind of visible cortical response as that obtained with sperm cells and other stimulating agents.

2. Typically the cortical response to non-electrolyte solutions results in the formation of a perivitelline space which is from two to three times as wide as that obtained upon insemination.

3. Continued exposure to non-electrolyte solution following the visible cortical response results in the dissolution of the fertilization membrane.

4. Since activation, however incomplete, is obtained upon treatment with non-electrolyte solutions, these solutions may be regarded as being artificial parthenogenetic agents.

5. Fertilization has been superposed upon the incomplete activation obtained in the presence of non-electrolyte solutions.

6. Within certain limits, as the length of exposure to the non-electrolyte solution increases, an increase in the percentage of blastulae among the inseminated treated eggs occurs; this fact may be correlated with (3) above.

7. It has been suggested that results reported by Moore (1930*a*, 1930*b*), Moore and Moore (1931), Motomura (1934, 1938), and Chase (1935) may be explained on the basis of the observations and results presented in this paper.

8. The amoeboid activity of *Arbacia* eggs treated with urea has been briefly described.

9. The observations and results presented in this paper are essentially in agreement with the views considered in the earlier papers of the present series (Moser, 1939*a*, 1939*b*).

LITERATURE CITED

Carter, G. S., 1924. On the early development of the echinoderm egg. II. The effects of changes in the surrounding medium on the initiation of development and on membrane-formation. *Proc. Camb. Phil. Soc. Biol., Sci.,* **1**: 84.

Chase, H. Y., 1935. The origin and nature of the fertilization membrane in various marine ova. *Biol. Bull.,* **69**: 159.

Churney, L., 1940. Mitotic elongation. II. Osmotic and salt effects. *Physiol. Zoöl.,* in press.

Glaser, O., 1913. On inducing development in the sea-urchin (Arbacia punctulata), together with considerations on the initiatory effect of fertilization. *Science,* N. S., **38**: 446.

Gray, J., 1922. A critical study of the facts of artificial fertilization and normal fertilization. *Quart. Jour. Micros. Sci.,* **66**: 419.

Harvey, E. B., 1939. Development of half-eggs of Chaetopterus pergamentaceus with special reference to parthenogenetic merogony. *Biol. Bull.,* **76**: 384.

Heilbrunn, L. V., 1937. An Outline of General Physiology. W. B. Saunders Co., Philadelphia.

Heilbrunn, L. V., and K. Daugherty, 1933. The action of ultraviolet rays on Amoeba protoplasm. *Protoplasma,* **18**: 596.

Just, E. E., 1919*a*. The fertilization reaction in Echinarachnius parma. I. Cortical response of the egg to insemination. *Biol. Bull.,* **36**: 1.

Just, E. E., 1919*b*. The fertilization reaction in Echinarachnius parma. III. The nature of the activation of the egg by butyric acid. *Biol. Bull.,* **36**: 39.

Just, E. E., 1922. Initiation of development in the egg of Arbacia. I. Effect of hypertonic sea-water in producing membrane separation, cleavage and top-swimming plutei. *Biol. Bull.,* **43**: 384.

Just, E. E. 1923. The fertilization-reaction in Echinarachnius parma. VI. The necessity of the egg cortex for fertilization. *Biol. Bull.,* **44**: 1.

Lillie, F. R., 1902. Differentiation without cleavage in the egg of the annelid Chaetopterus pergamentaceus. *Arch. f. Entw-mech.,* **14**: 477.

Lillie, F. R., 1919. Problems of Fertilization. Chicago.

Lillie, F. R., 1921. Studies of fertilization. IX. On the question of superposition of fertilization on parthenogenesis in Strongylocentrotus purpuratus. *Biol. Bull.,* **40**: 23.

Loeb, J., 1915. Concerning Brachét's ideas of the role of membrane formation in fertilization. *Biol. Biol.,* **28**: 87.

Marsland, D. A., 1936. The cleavage of Arbacia eggs under hydrostatic compression. *Anat. Rec.,* **67**: 38 (preliminary report).

Marsland, D. A., 1938. The effects of high hydrostatic pressure upon cell division in Arbacia eggs. *Jour. Cell. Compar. Physiol.,* **12**: 57.

Marsland, D. A., 1939. The mechanism of cell division. Hydrostatic pressure effects upon dividing egg cells. *Ibid.,* **13**: 15.

Moore, A. R., 1930*a*. Fertilization and development without membrane formation in the egg of the sea urchin, Strongylocentrotus purpuratus. *Protoplasma,* **9**: 9.

MOORE, A. R., 1930*b*. Fertilization and development without the fertilization membrane in the egg of Dendraster eccentricus. *Protoplasma*, **9**: 18.

MOORE, A. R., AND M. M. MOORE, 1931. Fertilization and development without membrane formation in the egg of the sea urchin (Paracentrotus lividus). *Arch. Biol.*, **42**: 375.

MOORE, C. R., 1916. On the superposition of fertilization on parthenogenesis. *Biol. Bull.*, **31**: 137.

MOORE, C. R., 1917. On the capacity for fertilization after the initiation of development. *Biol. Bull.*, **33**: 258.

MOSER, F., 1937. The effect of urea upon the surface of unfertilized Arbacia punctulata eggs (preliminary report). *Biol. Bull.*, **73**: 388.

MOSER, F., 1939*a*. Studies on a cortical layer response to stimulating agents in the Arbacia egg. I. Response to insemination. *Jour. Exper. Zoöl.*, **80**: 423.

MOSER, F., 1939*b*. Studies on a cortical layer response to stimulating agents in the Arbacia egg. II. Response to chemical and physical agents. *Ibid.*, **80**: 447.

MOTOMURA, I., 1934. On the mechanism of fertilization and development without membrane formation in the sea urchin egg, with notes on a new method of artificial parthenogenesis. *Sci. Rep. Tohoku Imp. Univ. 4th Series Biol.*, **9**: 33.

MOTOMURA, I., 1938. Effect of some salt solutions on the parthenogenetic membrane formation of sea urchin eggs. *Sci. Rep. Tohoku Imp. Univ. 4th Series Biol.*, **13**: 85.

SCHECHTMAN, A. M., 1937. Localized cortical growth as the immediate cause of cell division. *Science*, **85**: 222.